THE CORPUS LUTEUM OF *DASYURUS VIVERRINUS*, WITH OBSERVATIONS ON THE GROWTH AND ATROPHY OF THE GRAAFIAN FOLLICLE.

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(Communicated by Professor J. T. Wilson, M.B., Ch.M.).

(Plates vi.-xx.)

Introduction.

For the past seventy-five years the exact mode of origin of the structure known as "Corpus Luteum" has been the subject of dispute. The series of changes which take place in the wall of of the evacuated Graafian follicle and result in the formation of the corpus luteum has been studied in different animals with varying degrees of completeness. Considerable uniformity under-lies the process in those animals in which this particular question has been studied, but different significances have been attached by different observers to a series of changes essentially similar. Briefly, it may be stated that some maintain that the corpus luteum originates from the membrana granulosa of the Graafian follicle, whilst others refer its origin to the wall of the follicle or theca folliculi. The writer of this paper has attempted to throw light upon the subject by observing the growth of the corpus luteum in the ovary of a small marsupial found in abundance in various parts of Australia, namely, the Native Cat (*Dasyurus viverrinus*). So far as he is aware, this has not been previously done systematically, though isolated contributions have been made to the literature of the marsupial ovary.

This paper was originally submitted as a thesis for the degree of Doctor of Medicine of the University of Sydney, and the author begs to express his thanks to the Senate of the University for permission to publish the results of his investigation.

The collection of material and the obtaining of certain data made use of in this paper were done by Mr. J. P. Hill, D.Sc., F.L.S., Demonstrator of Biology in the University of Sydney, and well known as an authority on the processes of generation in the mar supial. To him the author tenders his sincere thanks for suggesting the undertaking of this research and for placing at his disposal a splendid series of ovaries in an excellent state of pre- servation.

The work was carried out during the year 1902 in the Anato- mical Department of the University of Sydney, and to Professor J. T. Wilson, its head, the author tenders his best thanks for placing the resources of the Department at his disposal and for his kindly interest throughout the progress of this investigation; and he has also to thank Mr. Louis Schaefer, of the same Depart- ment, for aid in producing the micro-photographs which illustrate this paper.

Short Review of the Literature.

The literature of this subject is extensive, being scattered through periodicals, reviews, text-books and essays, and extends over three-quarters of a century of time, so that no exhaustive review of it can be undertaken, apart from the fact that many of the contributions in question are inaccessible in Australia. For a more extended criticism of different authors' views, and for an excellent bibliography up to the year 1895, the reader is referred to Sobotta's paper "Uber die Bildung des Corpus Luteum bei der Maus" in the Archiv für Mikroscopische Anatomie, Bd. 47, 1896, and to contributions mentioned therein; also to an article by the same author in Merkel and Bonnet's Ergebnisse, Bd. 8, 1898; and to J. G. Clark's article in the Archiv für Anatomie und Physiologie, 1898. It will be necessary to mention here only those points which enable the reader to follow the discussion.

In the year 1827 appeared a treatise by von Baer on the origin of the mammalian ovum. He described the ovum in the interior of the Graafian follicle, and recognised the fact of its escape by rupture. He described also the wall of the Graafian follicle, com-

posed of membrana granulosa and theca folliculi, with its two component parts, the theca interna and theca externa. The main features of his description have been followed by all succeeding authors. He studied the corpus luteum, and attributed its origin to the inner layer or theca interna of the theca folliculi. He took this view on the grounds that the theca interna is thickened before the rupture of the follicle, and that this layer already possessed the characteristic yellow colour due to the so-called " lutein " granules. He is one of the few authors who studied the earlier developmental stages of the corpus luteum, and did not base his description on a study of corpora lutea near or during the stages of complete development.

No important communication was made upon this subject until 1840; when Patterson, of Edinburgh, brought forward a theory that the corpus luteum had its origin from the hæmorrhagic contents of the recently ruptured follicle. This theory gained the support of Henle, but was never generally accepted.

In 1842, Bischoff in his work "On the History of Development of Ova in the Rabbit" enunciated a new theory that the corpus luteum originated by modification of the cells of the membrana granulosa of the Graafian follicle to form the characteristic cells of the corpus luteum. A few years later, in ovaries of the dog, guinea pig and roe, he found confirmation of this theory. Bischoff studied all the stages of the formation of the corpus luteum both early and late, so that his work has a particular value. There are, therefore, excluding Patterson's, two different theories as to the origin and development of the corpus luteum. As time went on, defenders came forward for either theory. As adherents to von Baer's theory, we find Rokitansky, His, Spiegelberg, Kölliker, Slavjansky, Gegenbauer, Paladino, Nagel, Bonnet, Schottländer, Minot and others; whilst Pflüger, Luschka, Waldeyer, Call and Exner, and others have supported Bischoff's theory. Much of the work was not carried out upon a series of ovaries containing corpora lutea at all stages of development, so that it loses to a certain extent its value.

Up to the year 1895 it will not be necessary to further review the literature, except to state that Waldeyer held a view which was a compromise between the two theories. He considered that the epithelial element bore the greater part during the early development of the corpus luteum, but that, in the later stages, the theca folliculi had the greater share in its formation.

The year 1896 marks a distinct advance in the mode of study of this subject. Then there appeared from the pen of Sobotta a paper based upon an exhaustive study of the development of the corpus luteum in the mouse. All modern methods of studying this question must comply with the requirements that he has laid down. His most important contention is that the corpus luteum must be studied in all stages of its development, from its origin onwards, and not only in its stage of full development. In this research there have accordingly been chosen ovaries representing all the chief stages of corpus luteum formation, the stage of the development of the ovum and embryo being taken as a guide to the time which has elapsed since fertilisation of the ovum. This has the greatest advantages from a comparative point of view. Sobotta's paper appears to have awakened fresh interest in this question. He follows Bischoff's theory, and is supported by Heape, Stratz, Honore, Belloy, van der Stricht, Bonnet, Bouin, and van Beneden; whilst Nagel, Clark, Rabl, Kölliker, Doering, Paladino, Bühler and His have come forward as upholders of von Baer's theory. More extended criticism of these authors' views will be given when necessary in the following chapters.

MATERIAL AND METHODS.

Dasyurus viverrinus is a small marsupial, averaging when full-grown about the size and weight of a half-grown rabbit. Once a year it has a period of rut, during the months of May, June and July, and only then does the congress of the sexes take place. According to Hill, a varying period intervenes between coitus and the fertilisation of the ovum, so that the period of gestation is uncertain. It is probably about eight days. After gestation is completed, the embryo is transferred to the pouch. Six are usually found there at a time. They remain attached to the

maternal teats for a period of seven or eight weeks, whilst the period of lactation is prolonged to about four months.

For the purposes of this investigation the sexes were allowed access to one another, and the date of coitus noted. The females were killed at a varying period afterwards. After securing the blood supply, the internal and external genitalia were removed and the stages of ovum or embryo corresponding were noted. The parts were placed in picro-sublimate solution for twenty-four hours and then transferred to alcohols.

Some were also treated with picro-nitric-aceto-osmic solution. Picro-sublimate fixing fluids were found very satisfactory.

The ovaries were transferred to absolute alcohol, cleared in cedar oil, afterwards put into benzol for 24 hours at a temperature of 45° C., to which melted paraffin at 40° C. to 45° C. was gradually added. Finally, the ovaries were transferred to paraffin with the melting point at 50° C., in which they were left at that temperature for a period of two to four hours. This method of embedding gave uniformly good results.

Sections were cut, some with Minot's microtome and some with the rocking microtome. Some were 6 micros. thick, but the majority 10 micros. They were mounted serially and stained according to requirements with Glychæmalum, Glychæmalum with Eosin, Iron-hæmatoxylin, and van Gieson's stain after Glychæmalum. This last was found particularly good for micro-photographs, which were taken with Zeiss' apparatus.

This paragraph gives the particulars of the material employed. Other ovaries besides those mentioned below were cut, but these were considered to show the most representative stages of development of the corpus luteum. The stage of ovum or embryo is indicated in each case.

Ovary, Stage A.

Ripe follicles, some showing maturation spindle in the ovum.

Ovary, Stage B.

Taken immediately after rupture of the Graafian follicle, ova obtained from uterus before separation of the second polar body.

This Stage is referred to in this paper as the Polar-body Stage.

Ovary, Stage C.

Five days post coitum, ova in one- and two-cell stages.

Ovary, Stage D.

Ova in the sixteen- and thirty-two-cell stages, with unclosed blastodermic vesicles in some cases.

Ovary, Stage E.

Blastodermic vesicles, average diam. 1 mm.

Ovary, Stage F.

Blastodermic vesicles, average diam. 2·5 to 3 mm.

Ovary, Stage F_1.

Blastodermic vesicles, max. diam. 3·5 mm.

Ovary, Stage G.

Blastodermic vesicles, average diam. 3·5 to 4 mm.

Ovary, Stage H.

Blastodermic vesicles, average diam. 6·5 to 7 mm.   Showing primitive streak and head process.   Very large corpora lutea projecting on the surface of the ovary.   This is about the stage of full development of the corpus luteum.

Ovary, Stage K.

Embryo 5·75 mm. long; late uterine stage, about the seventh day.

Ovary, Stage L.

New born young, three hours old, in pouch.

Ovary, Stage M.

Very small ovary, young animals 105 mm. long, head length 45 mm.

DEFINITION OF TERMS.

It will tend to avoid confusion if, before proceeding further, there be obtained a clear conception of what is meant by certain terms as used in this paper.   All authors are not agreed as to the use of the terms " corpus luteum," " corpus luteum verum," " corpus luteum spurium vel falsum," and " corpus luteum

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atreticum." In this paper the term "*corpus luteum*" will be held to indicate the product of the changes which take place in the remaining structures of the Graafian follicle, after the extrusion and subsequent fertilisation of the ovum, that is, the " corpus luteum verum " of certain authors. Sobotta and others do not think that the fertilisation of the ovum is necessary, and maintain that the processes are the same, whether the ovum be fertilised or not. Waldeyer has lately insisted that the distinction must be given up, and it is probable that the process of corpus luteum formation in Dasyurus ovaries, whose ova are extruded, is the same whether fertilisation takes place or not. The term " *corpus luteum falsum* " or " *spurium* " will, however, for the present be applied as a term of convenience to those corpora lutea formed in ovaries whose ova are not fertilised. The term " *corpus luteum atreticum* " will here be taken to mean the corpus luteum which forms in an unruptured Graafian follicle —the " atresic follicle " to be described subsequently. Paladino has recently defined "true corpora lutea" as being those structures formed in ruptured follicles, and classified as "false" those formed in atresic follicles. Beigel is the only author who agrees with him. This is confusing, because the term " corpus luteum spurium " is often applied in man and animals to the corpus luteum which forms when pregnancy does not occur, although the ovum has been extruded. This variety of corpus luteum is distinct from the variety formed in atresic follicles. Playfair says that the difference between "true " and "false " corpora lutea in man is only in degree, whilst Dalton applied the term "false corpus luteum " to atresic corpora lutea sometimes found in human ovaries. In this paper the writer will adhere to the ordinary definitions, as explained above.

The term " corpus fibrosum " is used by various authors. It will be taken to mean the structure remaining after the degeneration of the corpus luteum, although Patenko extends this name to the connective tissue structure, which in some cases obliterates the atresic follicle.

THE PRIMORDIAL FOLLICLE.

Before entering upon the consideration of the primordial follicle itself, it will be necessary for the sake of completeness to describe shortly the appearance of the ovary macroscopically and microscopically. The organ in the period of its greatest size is of ovoid shape, measuring on an average 6 mm. by 5 mm. in its diameters. When the follicles are ripe they show as projecting bosses upon the surface. After their rupture the " stigma " can be seen, and soon also the corpus luteum, standing out as a yellowish-white structure against the general background of the ovary. In section, the organ shows the usual fibrous connective tissue stroma, containing ova in various stages of development. Near the periphery of the ovary, the fibrous tissue is condensed, and the surface of the organ is coated by a layer of cubical epithelium. A similar condensation of connective tissue is found round the larger primordial follicles and the more fully developed Graafian follicles. The smaller vessels are placed in these condensed areas of connective tissue, whilst centrally and elsewhere are found the larger vessels of the ovary, surrounded by a more rarefied stroma substance. Generally speaking, the young primordial ova lie in the peripheral condensed ovarian stroma, a little distance below the surface epithelium, and in the angles between the larger ova and follicles, where they approach the surface; whilst the older ova are not localised to any particular part of the ovary, many being superficial and many buried some distance beneath the surface (see fig. 2).

The ovum in its earliest recognizable stage (primordial ovum, see fig. 1) appears as a rounded cell placed in the condensed peripheral ovarian stroma, just beneath the epithelium of the surface. It is larger than the surrounding cells, and contains a finely granular cytoplasm which stains less deeply with hæmatoxylin than the neighbouring connective tissue elements. It is clearly distinguishable from the surrounding cells, and possesses a nucleus with a nucleolus, the nuclear chromatin being arranged peripherally near the nuclear membrane. At first there does not appear

to be any trace of the future vitelline membrane or envelope of the ovum. In the cytoplasm of this primordial ovum, usually on opposite sides of the nucleus, are to be seen two dark bodies situated in a clear space, possibly the "centrosomes," which later on disappear. The cells of the ovarian stroma adjacent to the primordial ovum do not at first show any peculiarity, but soon cells make their appearance, which have a circular arrangement round the periphery of the ovic cell. These cells are flattened and epithelial in type, with a definite nucleus; their characters are shown in fig. 1. These cells soon become sharply marked off from the ovum by a membrane, the vitelline membrane. They are not at first marked off from the connective tissue stroma externally, but later on a membrane forms, which is the early representative of the "Glashaut," "basal membrane," or "membrana propria" of authors. The cells between the two membranes become more cubical in form, and are the first representatives of the cells of the membrana granulosa. At a very early stage, therefore, there are formed the rudiments of the more important structures of the ovarian ovum, with its surrounding cellular and membranous structures. The subsequent development of the ovic cell does not concern us further. Briefly, it increases in size, it forms yolk granules, the bodies like centrosomes disappear, and the nucleus becomes excentric or even peripheral.

After a certain stage, the ova grow very little, and the subsequent changes involve the membrana granulosa, which had attained the form of a single layer of cells, placed between two membranes. This single layer of cells multiplies to become a zone of cells, two, three, then nine to twelve cells in thickness, with nuclei showing many karyokinetic figures. The cytoplasm of the cells is lightly stained and the walls are indistinct, whilst the most externally placed cells (adjacent to the basal membrane) are regularly placed, and suggest an epithelial arrangement. The same applies in some cases to the cells near the vitelline mem-brane. At the angles between the cells are often to be observed spaces, probably to be accounted for by the rapid cell-growth not leaving time for the intercellular angles to be filled. These

spaces are different from other spaces, walled in by cells arranged
in an irregular way, due to the liquefaction of the cell cytoplasm
after chromatolysis of the nuclei. Definite " corps vésiculeux,"
with cells radially arranged round them, as first described by
Call and Exner in the rabbit—the "Epithelvacuolen" of Flemming
—are not seen in Dasyurus, although in the larger primordial
follicles and in the young Graafian follicles there are found spaces
between irregularly arranged cells, showing in their interior an
ill-defined system of trabeculæ. These are probably the repre-
sentatives of the so-called " corps vésiculeux " of Call and Exner
in Dasyurus. Similar structures are found in atresic follicles.

The characters of the theca folliculi will be described more fully
later on, during the consideration of the ripe follicle. To the
whole structure, as above described, consisting of ovum, mem-
brana granulosa, and membranes with the theca folliculi, may be
applied the term " primordial follicle."

The Graafian Follicle—its Formation and Ripening.

The development of the primordial follicle has been described
in the preceding section. The next important event in its
history is the formation of cavities in the membrana granulosa.
This is brought about by liquefaction of the cell contents, follow-
ing upon a chromatolysis of its nucleus, a process which can be
observed to take place in several places at once, but progresses
more rapidly in some places than in others. By the ultimate
coalescence of these cavities the ovum is left in the centre of a
vesicular structure, lined by cells of the membrana granulosa and
connected to it by bands of cells, called " retinacula" (see fig. 4).
This structure is called the " Graafian follicle." Its cavity con-
tains fluid, formed probably by cell liquefaction and by secretion
from them, and by the infiltration of lymph into the cavity. This
formation of fluid goes on—evidently against pressure, other
spaces appear in the membrana granulosa, the follicle increases
greatly in size and becomes ripe.

THE RIPE GRAAFIAN FOLLICLE OF *DASYURUS VIVERRINUS.*

In determining what constitutes a ripe follicle in Dasyurus, one is guided by certain considerations.

Firstly, when the ovary of a non-pregnant female is examined macroscopically during the oestral period, the follicles which are ripe, or nearly so, are easily perceptible as prominent projections on the surface. They may be as many as twelve to fifteen in number. Microscopically, these projections are found to be due to large follicles which occupy the greater part of the section (see fig. 5), the larger vessels being placed centrally, and the stroma being diminished to a minimum, whilst in the angle between these large follicles are found young ova and follicles, some of which show signs of atrophy.

Secondly, from the microscopical characteristics of the ripe follicle, to be describedly presently, it is easy to tell whether it be ripe or nearly so. Sobotta says that the test of a ripe follicle is that the nucleus of the ovum must not be in the resting stage, but must either possess a maturation spindle or be preparing for its formation. This holds good also for Dasyurus.

A description of the ripe follicle necessitates the following subdivisions :—

1. Of the ovum.
2. Of the liquor folliculi.
3. Of the membrana granulosa.
4. Of the theca folliculi.

Firstly :—The ovum (see fig. 5) is ellipsoidal and surrounded by a thick envelope; its detailed description will be published later. It is surrounded by a " discus proligerus," and is placed usually towards the periphery of the follicle, and often near the site of the future rupture. The cells of the discus proligerus are of the same type as the cells of the membrana granulosa to be described presently, and the ovum with its discus is connected with the membrana granulosa by strings of similar cells—the " retinacula."

Secondly :—The liquor folliculi fills the follicle. In preserved ovaries it is represented by coagulum, staining fairly well with ordinary stains.

Thirdly :—The membrana granulosa forms the epithelial part of the follicular wall. It varies in thickness. At or about the stigmatic area it is thinned, and is only four or five, or even two or three layers of cells in thickness, whilst near the ovum it may be seven to ten layers of cells in thickness. The cells are small and compressed, with nuclei, some of which stain intensely with iron-hæmatoxylin, whilst others stain faintly. Mitotic figures are absent. Vacuoles are often present at the intercellular angles.

The membrana granulosa is bounded externally by the membrana propria (see fig. 6), which forms a basal membrane on which the cells of the membrana granulosa rest. This basal membrane is homogeneous, clear and refractile on section, and on its outer side lies the theca folliculi. It is present in most animals, swine being an exception. Its nature and origin are disputed ; probably it is an altered layer of the theca interna.

Fourthly:— The theca folliculi is, from the point of view of this paper, the most interesting of the structures surrounding the ovum. In all animals whose ovaries have hitherto been studied, the theca folliculi is described as being specialised into two layers, an outer fibrous layer called the " theca externa," and an inner " theca interna," whose cells are polygonal with rounded nuclei, and contain in their cytoplasm granules of the so-called " lutein" substance, and are separated from each other by a varying amount of fibrous material. In Dasyurus, this specialisation of the theca folliculi into theca externa and theca interna is extremely rudimentary. Indications of it are seen in the thecæ of primordial follicles (see figs. 6 and 7), in which there can be made out an outer theca externa of fibrous character, and an inner theca interna, with irregular flattened connective tissue cells, having oval nuclei with no karyokinetic figures, a granular cytoplasm, but no definite lutein granules, and no obvious intercellular substance. This layer contains the smallest blood vessels. As

the follicle ripens, this differentiation becomes practically indistinguishable, the theca interna being represented by an indistinct layer of flattened nucleated cells, next the membrana propria, and resembling very closely the theca externa.

When the follicle is ripe, the stroma of the ovary generally is scanty; it is seen between adjacent ripe follicles as a thin layer of fibrous tissue, showing occasional nuclei, and contains larger blood vessels for the supply of the follicles. The stroma of the ovary is also very thin in the neighbourhood of the stigmatic area, where rupture will take place (see fig. 5). In Dasyurus, therefore, the theca folliculi has a rudimentary theca interna, which in the ripe follicle is reduced to a minimum, and never approaches the condition described in other animals.

The Rupture of the Follicle and its Closure.

When the follicle has become ripe it bursts, diminishes in size, and the position of its rupture can be seen on the surface of the ovary. The surface epithelium has broken away, and the fibrous wall opens to allow the extrusion of the ovum with discus proligerus and most of the liquor folliculi. The blood vessels of the thinned ovarian stroma throw out blood which in Dasyurus is found constantly upon the surface of the ovary. Intra-follicular hæmorrhage is very exceptional in this animal, and if it occurs is very slight, never more than a dozen red corpuscles being found in a ruptured follicle. The ova were found in the oviduct, some unsegmented, some giving off the polar body, some in the one- and two-celled stages. With the release of intra-follicular pressure the walls of the follicle tend to become approximated, corrugated and thickened, whilst at the site of rupture the two opposed surfaces of the membrana granulosa come together and adhere, so that there is formed a plug of epithelial cells (Bouchon épithélial), which closes the follicle (see figs. 8-9). This closure is an early event in Dasyurus, and takes place within the first few hours, before there is any attempt at formation of the corpus luteum.

Coincidently with these processes in the membrana granulosa, certain changes go on in the theca folliculi and the basal membrane. This latter is corrugated, but at first remains intact. The blood vessels of the theca dilate, either as a cause or an effect of the follicular rupture, and occupy the corrugations of the follicular epithelium and membrana propria. The blood supply increases, the cavity of the closed follicle shrinks, and the ovary begins the formation of its corpus luteum.

THE EARLY CORPUS LUTEUM.

In the consideration of the disputed question of the origin of the corpus luteum, the crucial period of time is when the constituent parts of the wall of the closed follicle undergo their transformation to form the component parts of the corpus luteum. It is, therefore, necessary to study these changes in detail. This description is founded upon the appearances of the early corpus luteum, at the Polar-body Stage of the ovum, and in the segmentation stages up to thirty-two cells, and the unclosed blastodermic vesicle, corresponding to Stages B, C and D previously indicated. The period of time occupied by the ovum in passing through these stages is not yet definitely known.

The three constituents of the follicular wall are affected in marked degree, and simultaneously. The event which can be considered to form the turning point in the transition of the follicle into the corpus luteum, is the rupture of the hitherto intact membrana propria. This is effected by the growth inwards of the theca folliculi, which bursts through the membrana propria and sends its connective tissue sprouts towards the cavity of the follicle. The membrana propria becomes indistinct near the apices of these sprouts and is lost. Elsewhere it persists, and for a comparatively long time can be seen as a homogeneous membrane sharply marking off the theca folliculi from the cells of the former membrana granulosa even after their transformation into the characteristic cells of the corpus luteum (see fig. 10).

The connective tissue of the theca folliculi plays a most important part in the formation of the early corpus luteum.

was seen previously that immediately after rupture of the follicle the blood vessels of the theca folliculi become enlarged at various points round the circumference of the follicle. At these points a sprouting of the connective tissue takes place. The cells enlarge, their nuclei increase in size, and some show karyokinetic figures. A new formation of blood vessels takes place at the same time, and these new blood vessels, with the connective tissue cells round them, wedge their way through the membrana propria and cells of the membrana granulosa toward the cavity of the follicle. This process goes on at the various points so that the whole early corpus luteum is represented by a lobulated structure (see fig. 11).

Next, the connective tissue bursts through the membrana granulosa completely, and reaches the cavity of the follicle, which it fills with loose connective tissue cells with processes which join, and form a connective tissue framework that fills the cavity of the ruptured follicle. In some cases, besides the connective tissue cells which are triangular in shape, there are found larger rounded cells with one or two nuclei, a granular protoplasm and a distinct wall. These often lie free in the cavity, singly or in masses, and many of them are seen in close proximity to bands of young fibrous tissue or between the ordinary connective tissue cells. They are probably " fibroblast " cells (see fig. 11).

The rate of metamorphosis of the cells of the membrana granulosa into those of the corpus luteum varies in its rapidity. Generally it does not begin until the connective tissue irrupts into the cavity of the follicle. In a few cases the cells of the membrana granulosa undergo their metamorphosis, and almost completely fill the cavity before the connective tissue reaches it. At first the cells of the membrana granulosa, on being released from intrafollicular pressure, are crowded together by the collapse and corrugation of the follicular wall. The cells nearest the membrana propria and the irrupting blood vessels, are the first to show an alteration in character. Their cytoplasm swells and their nuclei become more regularly arranged than the nuclei placed more centrally. Many of them are oval in shape, and have the

long axis of the nucleus radially placed. This swelling of the cytoplasm pushes the more centrally placed cells inwards, so that they encroach upon the central cavity with its connective tissue trabeculæ, and insinuate themselves between these trabeculæ. This cellular change is of the nature of an hypertrophy. No multiplication of the cell nuclei by direct or indirect division can be made out, though carefully and often searched for in sections treated with different stains.

To sum up, the early corpus luteum is formed from the ruptured follicle by two processes; first, an invasion of the cavity of the follicle by vascular connective tissue sprouts of the theca folliculi, it being impossible to distinguish between the part played by the theca externa and the rudimentary theca interna; and secondly, by an hypertrophy of the cells of the membrana granulosa.

THE LATER DEVELOPMENT OF THE CORPUS LUTEUM.

For the study of the later development of the corpus luteum, serial sections were made of ovaries corresponding to the stages of ovum and embryo represented by closed blastodermic vesicles up to the time of the formation of the primitive streak and head process of the early embryo, that is the Stages E, F, F_1, G and H, previously described. The last of these stages of the embryo is reached probably about the third day, though this point has not yet been accurately determined, and represents approximately the time when the formation of the corpus luteum is complete.

The formation of the corpus luteum is carried on along the lines already described. It has been shown how the connective tissue invades the cavity in the interior of the ruptured follicle. The chief feature of the connective tissue during the later development of the corpus luteum is the formation in connection with it of blood vessels, so that the interior of the young corpus luteum becomes filled with an angiomatous structure formed by branching blood vessels whose walls are composed of a single layer of flattened endothelial cells. These vessels exist under usual circumstances for some time previously to the filling of the spaces

between them with corpus luteum cells. Whether they are formed by the connective tissue previously in the interior of the follicle, or by the sprouting of the blood vessels of the theca, is uncertain (see figs. 12, 13 and 14). These blood vessels are of the nature of venous sinuses, and no structures comparable to small arteries are found in the corpus luteum at any time.

Whilst the vessels are forming, the cells of the membrana granulosa undergo a peculiar change. This has been seen in its initial stages previously, but now becomes more marked. The cell cytoplasm swells, becomes filled with granules (even drops of secretion are described in some animals), the cell boundaries become distinct and the nuclei become oval with their long axis radial, whilst many of them have two distinct nucleoli. The nuclear chromatin becomes aggregated round the periphery of the nucleus, but karyokinetic figures are uniformly absent, though they have been searched for with great care. Some of the cells have two or even three nuclei, and the multiplication of the cells, if it takes place, is direct. Taken on the whole, the change in the membrana granulosa cell is probably a process of simple hypertrophy. The hypertrophying cells push inwards the other cells into the spaces between the blood vessels previously described, so that the cavity is encroached on and filled up. In most cases this filling up is completed at the Stage H, but a cavity filled with blood vessels and connective tissue may persist for a long time (see figs. 15 and 16).

The membrana propria loses its former distinctness. It is encroached upon by the cells of the theca nearest to it. The nuclei of the cells nearest the membrana propria increase in size, the membrana propria is dissolved, and the connective tissue cells invade the cells of the corpus luteum.

By the processes described it comes to pass that the emptied follicle is transformed into the corpus luteum. Briefly, these processes are the irruption of connective tissue into the cavity of the follicle and its subsequent vascularisation, accompanied by hypertrophy of the cells of the membrana granulosa. The corpus luteum forms quickly (within three days) and persists during the

greater part of the time that the animal is lactating, ultimately
disappearing when the young animal is capable of leading an
independent existence.

In addition to these changes involving the conversion of the
ruptured follicle into the corpus luteum, certain changes go on in
the neighbouring ovarian structures. In a general view of the
ovary in section, when the corpora lutea are formed it is found
that the whole section is occupied by these structures (see fig. 17),
whilst the general stroma of the ovary is reduced to a minimum.
During the ripening of the follicle and during the process of
corpus luteum formation the stroma becomes more and more
rarefied and more fibrous, probably owing to the diverting of the
blood supply to the nourishment of the follicle and the corpus
luteum. This rarefaction is due to the disappearance of some of
the connective tissue cells and the imbibition of fluid by the
connective tissues generally. During its progress the larger
vessels of the ovary and their branches between the corpora
lutea become filled with blood; this congestion of the ovary
during corpus luteum formation is a marked feature of the organ.
This rarefaction persists whilst the corpora lutea are present in
the ovary, and only with their disappearance does the ovarian
stroma resume its ordinary condition.

The theca folliculi is affected in the same way. It undergoes
changes in the later stages of corpus luteum formation. Its
external part participates in the changes of the ovarian stroma,
whilst its inner part (the rudimentary theca interna) has the size
of its cellular nuclei increased, and is best preserved in the places
where it is sending in its connective tissue processes towards the
centre of the corpus luteum.

In addition to the development of the corpora lutea, and the
changes in the stroma just described, other processes go on simul-
taneously in the ovary. If an ovary be examined at the time of
ripening of the follicles or shortly after they burst, it will be
found that it is crowded with young ova and follicles in various
stages of development (see fig. 2). The smallest and youngest
ova are found immediately below the surface, in the intervals

between the follicles, whilst the larger are often found at some distance below the surface. With the formation of the corpora lutea, most of these ova and follicles undergo degeneration, which seems to affect them all except the youngest near the surface, and those which are nearing ripeness. The ova between and close to the corporea lutea are the first to go, and the effect of the growing corpus luteum seems to make itself felt in ever widening circles, so that finally, when the corporea lutea are fully formed, nearly all the residual ova are atrophied, with the exception of those young ova immediately below the surface. A few of the larger ova, further away from the influence of the corpus luteum, persist for a little while longer, but ultimately they atrophy and become of no account. This change is a progressive one, and quickly shows itself, so that when the corpus luteum has attained its full development the ova, with the exception of the smallest and most superficial, are in a state of atrophy (see fig. 17).

The Atrophy of the Follicle, and the Corpus Luteum Atreticum.

In this place it will be fitting to describe the characteristics of atrophic follicles and the so-called "corpora lutea atretica," that is, the corpora lutea formed in connection with certain follicles whose ova are not extruded.

With regard to the atrophic or atresic follicle, it is found that its fate is not the same in all cases. The difference depends on the size and development of the follicle before its atrophy begins, and upon the degree to which atrophic and other changes go on in its component parts.

The fate of the follicles near their ripeness will be described later. The remaining unripe follicles may be roughly classified into two varieties, large and small, whose characters have already been sufficiently described. In the larger follicles, up to about a stage represented by fig. 4, the atrophic follicles may be divided into those which ultimately come to have left one layer of cells of the membrana granulosa, and those which have more than one layer, two or three being a usual number. The atrophic process

is similar in each case, and can be described as affecting, firstly, the contents of the follicles; secondly, the membrana granulosa; thirdly, the basal membrane; and fourthly, the theca folliculi.

The determination of the place where the atrophic changes begin is difficult. In Dasyurus the membrana granulosa is first affected. It will be seen that some of its nuclei stain deeply, and others only faintly. In these latter, chromatolysis takes place in the nuclei of those cells nearest the cavity of the follicle, the nuclear membrane disappears, and the chromatin becomes broken up into fine particles which are scattered through the general débris or aggregated into masses which stain deeply. Some of these deeply stained masses have been observed protruding from the cell nucleus, and in many cases seem to be nucleoli. In some cases also the formation of karyokinetic figures takes place in the nuclei of the cells of the degenerating membrana granulosa. This process has been fully described by Flemming and others, and is met with in atrophic follicles of many animals besides Dasyurus. Whilst this has being going on, the cell cytoplasm has undergone degenerative changes. In many animals a definite fatty degeneration has been described as taking place, but in Dasyurus this is not observed. The degeneration of cell substance appears to be a process of simple atrophy. It goes on until the membrana granulosa is reduced to a zone of cells inside the theca, often one or even two or three cells in thickness (see fig. 18).

The discus proligerus surrounding the ovum is not affected until comparatively late. Chromatolysis sets in, the cells atrophy in the usual way, and the envelope of the ovum crumples up, and is partially or totally destroyed, whilst its nucleus undergoes chromatolysis, and its cytoplasm loses its yolk granules and degenerates. By these processes the contents of the follicle come to be represented by a granular material which represents the liquor folliculi, together with débris of membrana granulosa cells and of the ovum. Through this granular material are scattered darkly stained granules, representing portions of the original chromatin of the various nuclei. Occasionally, too, cells are found, situated in a clear space in this granular content of the

follicles. These are leucocytes, with the function of removing the granular débris; and there does not, in Dasyurus, appear to be any reason for thinking that these are membrana granulosa cells with a phagocytic action, such as has been attributed to them by some authors, *e.g.*, Schulin, Janosik, and Pflüger. Whilst the ovum is degenerating similar cells are sometimes found attacking it, but nowhere is there seen any sign of the transformation of membrana granulosa cells into a plasmodium, with the phagocytic action, as described by Matchinsky, though in small atrophic follicles there are sometimes found appearances similar to those figured by Matchinsky, which are but remnants of degenerated ova containing nucleated leucocytes (fig. 21).

The basal membrane is early affected. It disappears whilst the above described changes are going on. It is generally an early event, but may be late. The time of its disappearance varies, but whilst it is present there is no tendency for the innermost layer of the theca folliculi to encroach on the membrana granulosa (see figs. 19 and 20).

When the basal membrane disappears, the rudimentary theca interna folliculi encroaches on the membrana granulosa. It becomes thickened, its cells increase in size and project into the membrana granulosa, and even into the cavity of the follicle. With the atrophy of the remaining membrana granulosa cells, there is also multiplication of the cells of the theca interna, so that concentric layers of connective tissue cells are formed, tending to diminish the size of the follicular cavity. This process is aided by the ingrowth of connective tissue cells into the cavity, causing its ultimate obliteration.

In the second variety of these atrophic follicles, where there remains but one layer of cells of the membrana granulosa lining the cavity, the ordinary separation of the rudimentary theca interna from the membrana granulosa by the basal membrane, though present, is obscured, and there is no attempt at encroachment on the membrana granulosa by the theca folliculi, so that the atrophied follicle presents the appearance of a cyst, lined by a definite layer of more or less cubical epithelium, towards which

the theca, at any rate for a time, may preserve its usual relations (see fig. 19). Occasionally, it appears that these cysts may remain for a considerable time, but the majority lose their layer of epithelium, and are obliterated by proliferation of the rudimentary theca interna and invasion of the cavity by connective tissue cells, as above described. It is probable that the two varieties of these larger atrophic follicles are due to variations of the same process, but the origin of the variation is obscure. The outer layer of the theca undergoes the changes which have been described previously in the consideration of the stroma of the ovary.

In the case of the smaller follicles, the process is less complicated, though similar. The vitelline membrane shrinks from the membrana granulosa cells, the ovum degenerates and is removed, whilst the membrana granulosa cells may persist as a single layer of cuboidal epithelium, or may atrophy, when the theca proliferates and fills up the cavity of the follicle. Occasionally, as in the case of other animals, a metaplasia of membrana granulosa cells into spindle- and star-shaped cells takes place. These fill up the space and cause its obliteration (see fig. 21).

In the case of follicles which are ripe or nearly so, whose ova are not extruded, there takes place a quite different process, which is not seen at all in the atrophy of the younger follicles. Practically, with the exception of the extrusion of the ovum, everything proceeds in the same way as if rupture had taken place. A corpus luteum atreticum is formed in the centre of which the atrophic ovum is seen, sometimes even making an attempt to segment (see figs. 22, 23, 24). The atrophied ovum is invaded by connective tissue and is removed by leucocytes. The membrana granulosa cells hypertrophy, the connective tissue of the theca grows in, in the same way as in the ordinary corpus luteum, and there is thus formed a corpus luteum atreticum.

It will be seen, therefore, that in Dasyurus there is no difference in the formation of the corpora lutea atretica, as compared with the mode of formation of the true corpus luteum. Some difference in size can sometimes be made out, the atresic being

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smaller than other corpora lutea, but, with this exception, the process is the same.

THE DECLINE OF THE CORPUS LUTEUM.

The growth of the corpus luteum in Dasyurus is rapid, and occupies the first three days after the follicular rupture. It remains in the same state for seven to eight weeks and then declines.

The chief factor in the decline of the corpus luteum is the supervention of a condition of fatty degeneration in its characteristic cells. The degenerated cells are removed by leucocytes, the blood vessels atrophy, and the connective tissue increases to form a corpus fibrosum, so that by the time the young animal is about ten centimètres long, some four months after its birth, there remains no trace of the corpus luteum in the ovary, which is found to be full of young ova beginning to grow in preparation for the next oestral period.

THE CORPUS LUTEUM OF *DASYURUS VIVERRINUS*.

In the short review of the literature given previously, the two main theories of origin of the corpus luteum were set forth. Some attribute its origin to the theca interna folliculi, others to the membrana granulosa. These divergent views have been taken by different authors for different animals, and in some cases the accounts differ for the same animal. It is worthy of note that those authors who have studied series of ovaries, sufficient to provide all the early stages of corpus luteum formation, are practically unanimous in contending that the characteristic cells of the corpus luteum take origin from the cells of the membrana granulosa. One of the best known of these is Sobotta, who, in the mouse and rabbit, carried out an exhaustive research on this question, and was the first to lay down the lines along which work to solve this question must be carried out. He is supported by Stratz, who in a lengthy article, including amongst others matters the history of the corpus luteum, gave a similar account of its origin founded on a complete study of numerous ovaries of *Tupaja*

javanica, Sorex vulgaris, and *Tarsius spectrum.* Honore, too, in the rabbit, working in the same way, arrived at the same general conclusions as Sobotta and Stratz; and van der Stricht, working on ovaries of *Vespertilio murinus, V. pipistrellus, Plecotus auritus,* and *Vesperugo noctula,* from which an abundance of material was obtained, confirms Bischoff's theory. Van Beneden in the rabbit, Belloy in the guinea pig and rat, Heape in the monkey, Bouin in the rat and guinea pig, Bonnet in the dog, and Cornil and Kreis in man, have all arrived at a similar conclusion, though differing in minor points. Of these authors, Sobotta, van der Stricht, Honore and Stratz may be taken as examples of those who have founded their support of Bischoff's theory on the study of a sufficient series of ovaries to give them all the stages of corpus luteum formation, particularly the early ones. On the other hand, it is found that the upholders of von Baer's theory, famous anatomists though some of them be, have studied this question in an imperfect way. His, Kölliker, Rabl, Nagel, Paladino, Clark, Doering, and Bühler have lately come forward as opponents of Bischoff's theory. With the exception of Bühler, whose work is not yet complete, none of them have carried out a study of the corpus luteum in all its stages of development, or at any rate there is no record of their having done so. Therefore their statements do not bear so much weight as they otherwise would. It is only fair to state that, with regard to His and Kölliker, their more recent remarks on this subject were made in short discussions at Anatomical Congresses. Rabl admits that his material is not sufficient to be of great service in settling this question, whilst Nagel's opinion is expressed but shortly in von Bardeleden's "Anatomie." Paladino's recent contribution to this question is founded on old observations made without respect to more recent requirements. In the case of Doering and Clark, they have collected swine ovaries (in large numbers, it is true), but without reference to any data as regards oestrum, time of coitus, and stage of pregnancy; or if any, so that they have no certain knowledge of the actual stages of corpus luteum they have described.

Next, it is interesting to note that the discrepancy of authors' views depends to some extent on the size of the animal studied. His, for instance, said at Kiel in 1898, that in man and in larger mammals, the formation of corpora lutea from the theca interna was absolutely indisputable. Bühler, too, at Pavia in 1900, said that it was certainly not an accidental circumstance that the accounts of the origin of the corpus luteum should be so diametrically opposed in the smaller as compared with the larger mammals. Sobotta (Tübingen, 1899) denies that the size of the animal has anything to do with the question, and upon the grounds of comparative anatomy there would appear to be no reason why the origin of such a constantly occurring structure as the corpus luteum should be so radically altered. If it is so, in what animals of the mammalian order does the transition in mode of formation take place, or where do the transitional forms of corpus luteum appear ? One is justified in supposing that the mode of formation of the corpus luteum is uniform throughout the mammalian order, though it may be obscured by accidental circumstances, so that, what holds good in Dasyurus—a marsupial—would probably hold good in the rabbit, mouse and guinea pig, and probably also in larger animals, as man and the swine.

But what are these accidental circumstances ? It has been pointed out by various authors that the theca folliculi of most animals is composed of an outer fibrous theca externa and an inner theca interna, whose cells are more or less polygonal and filled with granules of the so-called "lutein " substance, and have between them a certain amount of intercellular fibrous tissue. There is in fact a definite specialisation of the theca folliculi into two layers. Now, many authors attribute the origin of the characteristic cells of the corpus luteum to the specialised theca interna, some on altogether insufficient grounds. Thus His (at Kiel, 1898) says that the structure of the theca interna folliculi is identical with that of the young corpus luteum. Nagel uses the same argument, but that is no proof that one originates from the other. His also says that the transition from one to the other can be traced step by step. Does he refer to one corpus luteum, or to

the tracing of the transition through a series of ovaries containing
corpora lutea in all stages of development? In Dasyurus, as
shown previously, the specialisation of the theca folliculi is rudi-
mentary; in other words, the accidental specialisation of the
theca folliculi seems to be practically omitted, possibly owing to
the lowly position of the animal in the mammalian order. There
is accordingly no similarity between the cells of the rudimentary
theca interna and the cells of the membrana granulosa or corpus
luteum in Dasyurus, and consequently there is no mistaking the
parts these two structures play in forming the corpus luteum. In
tracing the transition, of which His speaks, in fully formed
corporea lutea of Dasyurus, a certain apparent resemblance is at
times to be seen between some of the theca interna cells and the
cells of the corpus luteum. A superficial observer might view
some of the cells as showing a transition of the theca interna
cells into the cells of the corpus luteum, but if the growth of
that structure is traced through its various stages it is found
that the rudimentary theca interna plays no part in the forma-
tion of the characteristic cells of the corpus luteum, but limits
itself entirely to the giving off of vascular connective tissue
sprouts to the interior of the follicle. And this is what is to be
expected, for the theca folliculi, from the time of formation of
the primordial follicle up to the rupture of the follicle, merely
plays the part of a stratum of tissue whose function is to provide
blood supply and support to the contents of the follicle. And
indeed, the function of the theca folliculi seems to have been
neglected in this connection by the majority of authors. In
most animals there is described a thickening of the theca interna,
with lutein granules in its cells during ripening of the follicle,
which has been considered by some as a process of preparation
for the formation of the corpus luteum by that layer. In Dasy-
urus, on the contrary, there are no cells containing lutein granules,
and during the ripening of the follicle there is a progressive
diminution of the rudimentary theca interna and a rarefaction
of the theca generally, so that it is extremely unlikely that the
sudden rupture of the follicle should bring about such a change

in the hitherto passive theca folliculi as to enable it to form a large parenchymatous cellular structure like the corpus luteum.

But, apart from these general arguments, a solution of the question for Dasyurus will be found on observation of the actual processes of corpus luteum formation in that animal, and perhaps the most important point in time is that, just after the rupture of the follicle takes place, when the process of corpus luteum formation is carried on with great rapidity, particularly in small animals. On this account, Bühler says that Sobotta has not observed in the rabbit and mouse the first alteration of these folliculi, in which sprouting of the thecal tissue into the cavity of the corpus luteum takes place; and he says also that an observer who is unaware of the existence of these sprouts or cones of thecal tissue might regard them as parts of the epithelial layer. This criticism is entirely erroneous, for in both the cases of the rabbit and mouse, under the sections dealing with the freshly ruptured follicle and early corpus luteum, Sobotta has described, though perhaps not fully, these cones of thecal tissue sprouting from the theca interna. Certainly in his figures this point might have been made more clear, but there is no doubt that he has both recognised and described them. To resume, in Dasyurus, owing to the comparative simplicity of the theca folliculi, it is easy to follow its future development. At first it does not transgress the membrana propria, but soon ruptures it and wedges its way towards the cavity of the corpus luteum, and even at this time before it reaches the cavity it can be seen in many cases that the changes in the cells of the membrana granulosa are well marked, whilst between the sprouts are seen the membrana granulosa cells still sharply separated from the theca by the basal membrane. In some instances, too, the cavity of the follicle is almost filled with the hypertrophied cells of the membrana granulosa, even before the vascular connective tissue sprouts from the theca have time to gain the cavity of the follicle. The rate of alteration in the membrana granulosa cells and in the theca folliculi is not absolutely constant, but varies within limits, so that at given stages of the segmenting ovum there may in one

case be found the corpus luteum a little further advanced than in another.

The amount of central tissue in the fully formed corpus luteum varies. Sometimes its interstices are completely filled with cells of the corpus luteum; at other times there persists for some time a cavity in the centre whose only content is connective tissue with occasional blood vessels.

In the preceding part of this section the chief points in connection with the theca folliculi have been shortly discussed. There are, however, in the case of the membrana granulosa other points concerning which authors are at variance.

In Dasyurus the membrana granulosa, unlike the theca folliculi, is throughout its existence characterised by active change. This is early evidenced by the multiplication of its layers, by the occurrence of karyokinetic figures in the membranæ granulosæ of the primordial and Graafian follicles, and by the general characters of their epithelial cells. It would not, therefore, be surprising to find that, after the follicular rupture, the membrana granulosa should persist and play a part in the formation of the corpus luteum. Many authors deny that this is so. Nagel, for instance, says that—in man—the membrana granulosa disappears absolutely. Bühler, too, in his preliminary note says the same, though at present his proofs are not forthcoming; whilst Kreis and others maintain that in man the cells of the membrana granulosa multiply or hypertrophy to form the characteristic cells of the corpus luteum. There is, therefore, a difference between observers who have taken man as the basis of their observations. Clark and Doering also deny that the membrana granulosa forms the characteristic cells of the corpus luteum. Clark says that (in the swine) a few of his preparations showed some epithelium, others almost none; and from this he concludes that the membrana granulosa completely disappears at the time of, or soon after rupture of the follicle. This is all the notice that Clark deems necessary to give to the description of a process whose study is so important to thoroughly understand this question, and he does not show a single figure to illustrate it. The haphazard way in

which Clark and Doering collected their material has already been commented upon; but apart from this, the words of Clark's description indicate clearly that he is describing the membrana granulosa of an atresic follicle. In Dasyurus the same wholesale disappearance of membrana granulosa is observed, but only in atresic follicles up to a certain stage of ripeness. Doering denies that his isolated human corpus luteum was an atresic one, but owing to the imperfection of his diagrams and the shortness of his description, it is impossible to contradict his somewhat categorical statements. Doering says, too, in the earlier part of his paper, that "in most cases" the membrana granulosa disappears. He omits to mention what becomes of it in the remainder. Kölliker has also lately expressed himself as of the opinion that Sobotta's explanation of the origin of the corpus luteum in the mouse and rabbit does not convince him. He does not mention any of his own researches on the formation of true corpora lutea in the mouse, but on the ground that, in his opinion, they are of the same nature as corpora lutea atretica, he defends von Baer's theory.

Against these opinions we must weigh those of such authors sa Sobotta, Stratz, Honore, and van der Stricht, who have recently studied the corpus luteum in all its developmental stages, and described it with great care. They and many others are unanimous in their support of Bischoff's theory. It is unnecessary to dilate further on their mode of work, but this alone, in contrast with the comparatively scanty observations made by the defenders of von Baer's theory, entitles their opinion to the greater weight.

Turning to the membrana granulosa of Dasyurus, immediately after follicular rupture there is no sign of degeneration or disappearance of the membrana granulosa, and there has been seen and depicted the actual hypertrophy in the cell substance. It has been observed to begin nearest the theca, in many cases, even before the connective tissue has reached the cavity of the follicle. It has also been observed that it is usually well marked before the membrana propria loses its distinctness, and it is

constantly found close to the connective tissue ingrowths from the theca into the cavity of the rudiment of the corpus luteum, and near sources of good blood supply. Also, in the many hundred of sections of corpora lutea examined at all stages of their formation, there has not been seen in one single instance any sign of atrophy of the former cells of the membrana granulosa, no chromatolysis, no fatty degeneration nor other degenerative phenomenon. On the other hand, there is, as described previously, a vigorous and rapid hypertrophy of the membrana granulosa cells; and this, too, in an animal whose theca interna is absolutely unimportant, and limits itself to supplying vascular connective tissue to the corpus luteum.

Finally, though many authors agree as to the persistence of the membrana granulosa cells, there are differences of opinion as to their subsequent life-history, The disputed point is, whether the cells increase by a pure hypertrophy, or whether they actually multiply. Some authors describe karyokinetic figures as occurring rarely, or frequently, in the nuclei. These are :—van der Stricht in the bat; Belloy in the rat and the guinea pig; Stratz in Tupaja, Sorex and Tarsius; Bouin in the rat and guinea pig, and Kreis in man. On the other hand :—van Beneden in bats, Honore in the rabbit, and Sobotta in the mouse and rabbit, deny that there is any karyokinesis in the cell nuclei. In Dasyurus, after a pro- longed search, there has been found no karyokinesis. The nucleus seems to participate with the cell in its hypertrophy. Sometimes to all appearances one cell has two or even three nuclei. Possibly amitotic division of cells may occur, but this has also been sought for and not found.

Lately, some authors have concerned themselves with the structure of the individual cells. Regaud and Policard maintain that, with special staining, there can be demonstrated in the cells of the corpus luteum of the hedgehog droplets of a substance which may be supposed to be a cellular secretion, and this observation fits in with Prenant's hypothesis of the glandular nature of the corpus luteum.

To sum up, in *Dasyurus riverrinus* the process of corpus luteum formation is plain. In this process there are two factors which go on side by side. These are—firstly, the hypertrophy of the membrana granulosa cells to form the characteristic cells of the corpus luteum ; and secondly, the invasion of these masses of hypertrophying cells by a vascular connective tissue framework which supports and nourishes the whole structure.

The Functions of the Corpus Luteum.

Up to the present time several different theories have been brought forward to explain the functions of the corpus luteum; and Minot says "Concerning the function of the corpus luteum we possess scarcely any knowledge."

Most of the theories have had a mechanical basis. The corpus luteum has been deemed to act as a "stop-gap" to fill the cavity of the ruptured follicle, and thus to restore the circulatory conditions which have been disturbed by a sudden release in the tension of the ovary.

Clark offers another explanation. He maintains that the corpus luteum has the function of giving blood vessels to a part, which in the ordinary course of events would become scar tissue, and thus the ovary is maintained in a soft and resilient condition, favouring the complete development of future follicles.

Ingenious as this theory is, it seems that it, or any mechanical theory must be insufficient, which neglects to take into account the vital processes of the characteristic cells of the corpus luteum themselves, particularly in their relations to the changes in the rest of the ovary, in the uterus and genital apparatus, and in the whole organism generally. Prenant, in a highly interesting paper, has brought forward a very attractive theory to explain the origin of the corpus luteum (which he attributes to the membrana granulosa). He points out that, in all animals examined, the corpus luteum is a structure whose morphological characters are those of a glandular apparatus without a duct, possessing presumably an internal secretion; and that the cells of the corpus luteum elaborate material in their interior as has

recently been described by Regaud, Policard and others, and also that they do not show (except occasionally) mitotic figures, though on this last point authors disagree. The corpus luteum of Dasyurus comes into line with other corpora lutea in these respects, and, like them, resembles closely the liver in its histological features. Prenant considers the corpus luteum a gland, and he is of opinion that its hypothetical secretion plays an important part in the organism, such as we are accustomed to attribute to the supposed ovarian internal secretion. After attempting to explain the phenomenon of chlorosis by the lack of this possible internal secretion, he proceeds to argue that the purpose of the corpus luteum is probably also to prevent ovulation in the period between successive oestra, or during pregnancy. This latter theory is supported by Beard, Regaud and Policard; and in the case of Dasyurus can be supported on the following grounds :— On taking a general view of the ova and their intraovarian history in Dasyurus, it was observed that, in common with other animals, during the period between the oestra, and towards the end of lactation, the corpus luteum disappeared, and then the young ova began to grow in preparation for the next oestral period. Also, as soon as the corpus luteum is formed, it is found that the ova, hitherto in various active stages of development, begin to atrophy as described above. This atrophy begins in the neighbourhood of the young corpus luteum, and the process seems to affect the ova in ever widening circles. This atrophy may be due partly to mechanical pressure and partly to the internal secretion of the corpus luteum, if it has one. It is at any rate certain that, in Dasyurus during the time of development and persistence of the corpus luteum, atrophy of the larger remaining ova takes place, and ovulation remains at a standstill. Some of the remaining ova (exceptions to the rule) have at first sufficient energy to carry them on for a while, but ultimately the corpus luteum triumphs over them and they atrophy.

In this connection, Fraenkel and Cohn's experiments (see Anat. Anz. 1902, pp. 294-300) are of interest. Working on Born's theory, that the corpus luteum is a gland elaborating an internal

secretion whose function was to prepare the uterus for the reception of the egg, and to give the impulse to the organismal changes accompanying pregnancy, they performed certain experiments, suggested by the following considerations :—

(1) That the ovum cannot itself produce the changes in the organism, for these begin even before the ovum reaches the uterus.

(2) In ectopic gestation the uterus undergoes the usual changes, although the ovum is in the tube.

(3) If the ovum reaches the uterus, its growth alone does not explain the great increase in size of the uterus, and there must be some other factor at work.

The corpus luteum was thought to be this factor, because it is a large structure whose function is not evident, and which is remarkably constant throughout the mammalian order. These observers then, as an additional reason, say that Aplacentalia, such as Monotremes and Marsupials, whose ova develop outside the uterus (sic) possess only a rudimentary corpus lutem, or none at all. This is erroneous. Both these classes of animals have a large corpus luteum, consequently this reason carries no weight.

By their experiments they claim to have shown that, in the rabbit, destruction of the corpora lutea prevented the ovum from being retained in the uterus, and that the presence of the corpus luteum has some influence on this retention. Their work is not concluded, but promises to throw some new light on the function of the corpus luteum.

Some may object that these arguments lose weight in the case of the false corpora lutea and the corpora lutea atretica. But a similar, though modified, explanation may be considered to hold good in these cases. The ovary, in the case of the false corpus luteum, does not concern itself with the fate of the ovum. The absence of fertilisation can be considered to be an accidental failure of Nature's intention. The corpus luteum forms in just the same way, and with the same effect of staying ovulation until it atrophies, and possibly with the intention of preparing the genitalia and the organism generally for the changes which would

under ordinary circumstances ensue. With regard to the corpus luteum atreticum, the failure of extrusion of the ovum is an accidental departure from the normal, so that in the case of follicles which have attained a certain stage in their process of ripening, they possess sufficient energy to proceed with the formation of a corpus luteum atreticum, thus trying to carry out their function. And here it is interesting to observe that occasionally the ovum of the atresic follicle itself undergoes a kind of parthenogenetic division, which seems to be an attempt on its part to carry out its destiny.

To sum up, it may be stated as probable, firstly, that the corpus luteum is a glandular structure with an internal secretion; and secondly, that it influences the genital organs and the organism generally and prevents ovulation during pregnancy, and temporarily if pregnancy does not occur.

SUMMARY.

The chief conclusions arrived at in this investigation on the corpus luteum of *Dasyurus viverrinus* are :—

(1) The characteristic cells of the corpus luteum are formed by hypertrophy of the cells of the membrana granulosa.

(2) The theca interna folliculi is rudimentary and forms only the vascular connective tissue of the corpus luteum.

(3) The corpus luteum atreticum is formed in the same way as the corpus luteum verum.

(4) Other atresic follicles are reduced to fibrous tissue or remain cystic.

(5) The corpus luteum is probably a gland with an internal secretion of use in the organism. It has the function of stopping ovulation during pregnancy and at the oestral periods.

BIBLIOGRAPHY FROM 1895 ONWARDS.

AZEVEDO NEVES.—" Contribucao para estudo do ovario." Thesis. Lisbon, 1901 (cited by Limon, v. i.).

BEARD, J.—(1) "Rhythm of reproduction in Mammalia." Anat. Anz. Bd. 14, 1897, pp. 97-102.
———————(2) " The Span of Gestation and The Cause of Birth."

BELLOY, G. — "Recherches sur l'origine des corps jaunes de l'ovaire chez le rat et le cochon d' lnde." Compt. Rend. Assoc. Anat. (Nicolas) Paris, 1899.

VAN BENEDEN.—E. (für HONORE) "Ueber Corpus Luteum beim Kaninchen." Verh. d. Anat. Ges. Tübingen, 1899.

BOUIN, P. & M.—"A propos de follicules de de Graaf, follicules polyovulaires," &c. Compt. Rend. Soc. Biol. Paris, T. 52.

BOUIN, P.—(1) "Figures caryocinetiques des cellules des corps jaunes de la ovaire du cobaye." Compt. Rend. Soc. Biol. Paris, T. 5, pp. 163-164.

—————-(2) "Atrésie des follicules de de Graaf, et formation de faux corps jaunes." Bibl. Anat. 1899.

BUHLER, H.—(1) "Die Rückbildung der Eifollikel bei Wirbeltieren." Morphologisches Jahrbuch, Bd. 30-31.

—————-(2) "Entwickelungsstadien menschlicher Corpora Lutea." Verh. d. Anat. Ges. Pavia, 1900, pp. 150-154.

BUHLIUS & KRETSCHMAR.—"Angiodystrophia Ovarii," 1897. (Stuttgart) quoted by Rabl. Anat. Hefte, 1898, pp. 127 et seq.

CLARK, J. G.—"Ursprung, Wachsthum, und Ende des Corpus Luteum, nach Beobachtungen am Ovarium des Schweines und der Menschen." Archiv für Anat. und Phys. 1898.

CORNIL.—(1) "Note sur histologie du corps jaunes des femmes." Annal. de Gynak. et Obst. Année 26, T. 52, pp. 373-381.

—————(2) Bull. et Mém. de la Soc. Anat. Paris, Année 74, T. i., 1899, pp. 653-664.

DOERING, H.—"Beitrag zur Streitfrage über die Bildung des Corpus Luteum." Anat. Anz. Bd. 16, 1899.

EBNER, V.—Kölliker's 'Handbuch der Anatomie.'

FRAENKEL & COHN.— "Experimentelle Untersuchungen über den Einfluss des Corpus Luteum auf die Insertion des Eies." Anat. Anz. Bd. 20, pp. 294-300. The former also in Archiv für Gynäk. 1902. Bd. 68, H. 2.

HEAPE, W.—"Ovulation of Macacus rhesus, &c." Phil. Trans. Vol. 188, 1897.

HILL, J. P.—"Foetal Membranes of Dasyurus viverrinus, &c." Anat. Anz. Bd. 18, pp. 364 et seq.

HIS, W.—Discussion. Verh. d. Anat. Ges. Tübingen, 1899.

HONORE, CH.—(1) Per E. van Beneden—Discussion. Verh. d. Anat. Ges. Tübingen, 1899.

—————-(2) "Recherches sur l'ovaire du lapin." Arch. de Biol. T. 16, pp. 537-601; T. 17, pp. 489-499.

JANOSIK.—" Die Atrophie der Follikel und ein seltsames Verhalten der Eizelle." Arch. mikr. Anat. Bd. 48, 1896.

KREIS, O.—"Entwickelung und Rückbildung des Corpus Luteum Spurium bei Menschen." Arch. Gynäk. Bd. 58, 1899, H. 2, pp. 411 et seq.

KOELLIKER, A. VON.—(1) " Ueber die Entwickelung der Graafschen Follikel." Sitzungsber. der phys.-med. Ges. zu Würzburg, 1898.

———————————(2) " Ueber Corpora Lutea Atretica bei Säugetieren." Verh. d. Anat. Ges. Kiel, 1898.

———————————-(3) " Erinnerungen aus meinem Leben," pp. 299-301.

LIMON, M.—"Les vacuoles de la granulosa des follicles de de Graaf." Bibl. Anat. T. 10, 1902, p. 453.

MARSHALL, F. H. A.—"The oestrous cycle and formation of the Corpus Luteum in the sheep." Proc. Roy. Soc. Lond. Vol. 68, pp. 135-140; also Phil. Trans. 1903, Vol, 196.

MATCHINSKY.—"De l'atrophie des follicules dans les ovaires des mammifères." Ann. de l'Institut Pasteur. T. 13, 1900, pp. 113 et seq.

NAGEL, W.—Article "Die weibliche Geschlechtsorgane " in the " Handbuch der. Anat. herausg. von K. von Bardeleben," Bd. 7, T. 2, Abt. 1, Jena 1896.

PALADINO.—(1) " A propos de la question controversée relative à l'essence du corps jaune." Arch. Ital. de Biol. T. 34, p. 228.

———————- (2) " Per la diabattuta questione sulla essenza del Corpo Luteo." Anat. Anz. Bd. 17.

POULTON, E. B.—" The structures connected with the ovarian ovum of Marsupialia and Monotremata." Quart. Journ. Microscop. Sci., 1884.

PRENANT, A.—" La valeur morphologique du corps jaune, &c." Rev. gén. des Sciences pures et appliquées, 1898, pp. 646-650.

RABL, H.—(1) " Beitrag zur Histologie des Eierstocks des Menschen und der Säugetiere." Anat. Hefte, Bd. 11, 1898, pp. 109, et seq.

———————(2) " Mehrkernige Eizelle und mehreiige Follikel." Arch. mikr. Anat. Bd. 54, 1899, pp. 439, et seq.

REGAUD AND POLICARD—(1) " Notes histologiques sur l'ovaire des mammifères." Compt. Rend. Assoc. Anat. 3me Sess., 1901, pp. 45-61.

———————————(2) " Fonction glandulaire de l'épithélium ovarique chez la chienne." Compt. Rend. Soc. Biol. Paris, T. 53, 1901, pp. 615-616.

———————————(3) " Phénomènes secrétoires, formations ergastoplasmiques et participation du noyau à la secrétion dans les cellules des corps jaunes, chez l'hérisson." Compt. Rend. Soc. Biol. Paris, T. 53, pp. 470 et seq.

SOBOTTA, J.—(1) "Ueber die Bildung des Corpus Luteum bei der Maus." Anat. Anz. Bd. 10, 1895. Also Arch. mikr. Anat., 1896, Bd. 47.

————(2) "Ueber die Bildung des Corpus Luteum beim Kaninchen." Anat. Hefte, xxvi. .

————(3) "Noch einmal zur Frage der Bildung des Corpus Luteum." Bd. 53, 1898.

————(4) "Ueber die Entstehung des Corpus Luteum der Säugetiere." Merkel and Bonnet's 'Ergebnisse.' Bd. 8, 1898, and Bd. 11, 1901.

————(5) "Uber das Corpus Luteum der Säugetiere." Verh. d. Anat. Ges. Tübingen, 1899.

STRATZ, C. H.—" Der geschlechtsreife Eierstock." Haag, 1898.   Review in Schwalbe's Jahresberichte, Bd. 5, 1899, Abt. 3, p. 404.

VAN DER STRICHT.—(1) "La rupture du follicule ovarique et l'histogenèse du corps jaune." Compt. Rend. Assoc. Anat. 3me Sess., pp. 33-40, 1901.

————(2) "L'Atrésie folliculaire des follicules de de Graaf dans l'ovaire de chauve-souris." Verh. d. Anat. Ges. Bonn, 1901, pp. 108-124.

WALDEYER.—(1) "Das Becken." Bonn, 1898.

———— (2) "Normales Ovarium einer 45-jährigen Frau, mit zwei grossen Corpora Lutea." Verh. d. Anat. Ges. Tübingen, 1899.

EXPLANATION OF PLATES.

Plate vi.

Fig. 1.—Section of ovary (C) showing earliest stages of ovum (× 350).

Above and to the left is seen the surface epithelium of the ovary, and in the right hand corner below appears a portion of an early corpus luteum. Five young ova are seen in the ovarian stroma.   Round them all, cells are arranged more or less regularly.   These are the early representatives of the membrana granulosa.   The two smallest ova show no vitelline membrane; the other three do.   All the young ova show the granular character of the cytoplasm, two show nuclei, and one a definite nucleolus in the nucleus. Round the largest of the five ova, the membrana granulosa is seen to be two cells thick in the lower and left half, whilst above and to the right there appears a rudimentary basal membrane.   The rudiments of a theca are also seen outside the lower half of the membrana granulosa of this largest ovum.

Fig. 2.—Section of ovary (C) showing stages subsequent to those shown in fig. 1 (× 75).

Ova of various sizes are shown, and the multiplication of the layers of the membrana granulosa is seen.   Below in the right hand corner is a large ovum

with yolk granules, and a thick vitelline membrane, outside which are the basal membrane and the theca folliculi. A similar ovum is seen in fig. 3.

Plate vii.

Fig. 3.—Section of a primordial follicle from ovary C just before the appearance of the cavity (× 130).

Shows the ovum, containing yolk granules, with a thick vitelline membrane, and the cells of the membrana granulosa external to the vitelline membrane. Externally to the membrana granulosa again is a very definite basal membrane, and outside that the theca folliculi. A portion of the same follicle is shown under higher magnification in fig. 7.

Fig. 4.—A Graafian follicle at an intermediate stage of development (× about 75).

The ovum is seen in the centre, surrounded by a layer of cells which are attached by retinacula to the membrana granulosa. The basal membrane and theca folliculi can also be made out.

Plate viii.

Fig. 5.—Section of ripe follicle from the ovary, Stage A (× about 40).

This follicle was ripe as indicated by the maturation spindle in its contained ovum. The ovum is seen as an ellipsoidal body placed in the follicle, close to the surface of the ovary, and bound to the membrana granulosa by retinacula of cells. It is surrounded by the discus proligerus. The cavity of the follicle is partially filled with coagulum of the liquor folliculi. The membrana granulosa is shown and the theca externally.

Fig. 6 shows under high power portion of walls of adjacent ripe follicles (× 350).

This figure and the next indicate well the characters of the theca folliculi. Above and below are the membranæ granulosæ of the ripe follicles, some of the nuclei being faintly and others darkly stained. Indications of "Epithelvacuolen " are seen in places. The membranæ granulosæ are set upon a distinct membrana propria or basal membrane. On the side of the basal membrane, away from the membranæ granulosæ, there are to be seen darkly stained nuclei of the cells of the theca interna, whose rudimentary character is well shown. The rest of the tissue between the membranæ propriæ is theca externa whose fibrous tissue characteristics are well shown. Two blood vessels, one filled with coagulum and the other with blood corpuscles, are seen in section, and between their overlapping ends is seen a small ovum.

Plate ix.

Fig. 7.—Portion of primordial follicle shown in fig. 3 (× 500).

This figure shows at the extreme top a portion of the ovum, and its vitelline membrane. Next comes the membrana granulosa, with its outer-

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most cells set upon a distinct membrana propria or basal membrane. Immediately outside (*i.e.*, below) the membrana propria is seen the theca folliculi, and this figure shows the most extreme specialisation of theca folliculi into theca interna and theca externa found in Dasyurus. Small blood vessels are seen in the theca, and the characters of the two parts of the theca are to be distinguished. The lower part of the figure is occupied by cells of the young corpus luteum. In one place is illustrated the tendency for the theca interna to send in a process of itself towards the central cavity of the corpus luteum (not shown in the figure but placed below). On either side of this process the basal membrane is still fairly distinct, and the metamorphosis of the cells of the former membrana granulosa into those of the corpus luteum is just commencing.

Fig. 8.—Portion of a section from ovary, Stage B (× 80).

This section was taken through a newly ruptured follicle, but the plane of section does not go through the site of rupture. It shows the corrugation of the membrana granulosa and the dilatation of the blood vessels of the theca, which are pushing portions of the membrana granulosa towards the cavity, in which some remnants of coagulum are left.

Plate x.

Fig. 9.— From same ovary, Stage B (× 80).

Shows the freshly ruptured follicle, which has been closed. The site of rupture is easily distinguishable and also the plug of cells closing the aperture (Bouchon Epithélial). The membrana granulosa is beginning to thicken. The blood vessels of the theca are seen to be dilated in several places, and in one portion of the follicular wall below and to the left between dilated blood vessels can be seen still the persistent membrana propria. The theca folliculi is seen best on the left hand side of the follicle.

Fig. 10.—Portion of the wall of an early corpus luteum from ovary, Stage B (× 350).

This figure shows a sprout of connective tissue projecting into the membrana granulosa. The membrana propria is still seen distinctly in one place. To its left are cells of the membrana granulosa, and to its right the tissues of the theca interna, containing blood vessels filled with red corpuscles.

Plate xi.

Fig. 11.—Section of ovary, Stage C (× 130).

This figure is extremely interesting. It shows the filling of the central cavity of the corpus luteum with connective tissue which is irrupting in several places, notably above and to the left.* Numbers of rounde dcells, probably " fibroblasts," are seen in the interior of the follicle, many of them free and others placed in juxtaposition to a strand of connective tissue,

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* The right side of the Plate is to be regarded as the top of the figure.

which stretches across the cavity of the young corpus luteum. The thicken-ing of the former membrana granulosa by hypertrophy of its cells is beginning, and in places, especially on the left of the section, the former relation of theca, basal membrane, and membrana granulosa still persists. The lobu-lated character of the young corpus luteum is also very apparent.

Plate xii.

Fig. 12.—From ovary, Stage D (× 40).

A slightly later stage of the corpus luteum. The cavity is practically filled with young connective tissue.

Fig. 13.—From ovary, Stage E (× 50).

Shows corpus luteum at later stage still. By this time the cells of the mem-brana have undergone part of their metamorphosis, and have extended further into the cavity. Blood vessels are also seen working their way in between the cells of the corpus luteum; the lower part of the cavity shows some blood vessels streaming in towards the centre of the cavity, whilst above are seen, in between the loosely arranged cells of the corpus luteum, larger venous sinuses filled with blood.

Plate xiii.

Fig. 14.—From ovary, Stage F (× 50).

Shows a slightly later stage than fig. 13. The centre of the corpus luteum is filled with connective tissue, and the blood vessels have also reached the centre. The corpus luteum cells have by this time assumed more definite characters, but have not yet filled the central cavity.

Fig. 15.—From ovary, Stage G (× 130).

Showing the characters of the cells of the corpus luteum. Running up the centre of the figure is a connective tissue ingrowth, and on each side cells of corpus luteum. Between many of the cells are seen intervals which are vascular spaces lined by endothelium.

Plate xiv.

Fig. 16 —From ovary, Stage H, showing characters of cells of fully formed corpus luteum (× 500).

Darkly stained nuclei of connective tissue are seen in various places; and in the centre of the figure an elongated vascular space, immediately against which on the left are placed the characteristic cells of the corpus luteum. The nuclei and nucleoli of individual cells are to be made out, and the cell boundaries are in some cases fairly distinct.

Plate xv.

Fig. 17.—Section of ovary, Stage H (low magnification). Five fully developed corpora lutea are shown. The ovarian stroma is scanty and

rarefied, and numbers of atrophic follicles are seen in various places. The dark lines in the corpora lutea themselves represent vascular spaces filled with blood, and darkly stained with hæmatoxylin. A few large blood vessels are also seen in the centre of the ovary.

Fig. 18.--An atresic follicle in an early stage of degeneration (× 80)

Note that the retinacula are dissolved, and that the interior of the follicle is partly filled with coagulum, containing small darkly stained granules. In the original specimen the basal membrane was still distinguishable outside the atrophic membrana granulosa.

Plate xvi.

Fig. 19 shows one large and two small atresic follicles (× 50).

The large follicle has the remnant of the ovum, with some of the coagulum of the liquor folliculi for its contents. The cells of the membrana granulosa are degenerating, and the basal membrane has disappeared. The two smaller atresic follicles show the ovum in the centre, and a single layer of epithelium, set upon a basal membrane, which is distinct in places.

Fig. 20.—An atresic follicle (× 130).

This figure shows the degenerated ovum and coagulum in the cavity of the follicle. It also shows the degenerating membrana granulosa indistinctly separated (in the upper half) from the theca folliculi. The rarefaction of the ovarian stroma is also well shown (below and to the right). The rest of the figure is occupied by corpora lutea.

Plate xvii.

Fig. 21 shows a number of atrophic follicles with portions of two young corpora lutea (× 130).

Above is seen the surface epithelium of the ovary. A little below are seen three atrophic follicles, the middle one being the most interesting. It shows centrally a lightly stained mass with darkly stained spots, the whole having the appearance of a plasmodium under the microscope. The mass represents the remnant of the ovum invaded by leucocytes. Immediately external to the mass is a zone of darkly stained cells, which under a high power are seen to be somewhat fusiform, and resemble cells of connective tissue. These were originally membrana granulosa cells which are probably undergoing a metaplasia, as described in the text. Outside this zone of darkly stained cells (seen best above) is a zone of cells which represents the theca folliculi. Portions of young corpora lutea are seen below, and three old atrophic follicles in the interval between.

Plate xviii.

Fig. 22 shows sections of three corpora lutea, two of which are "true," and one (the central) is a corpus luteum atreticum (× 50).

A similarity in the general arrangement of the cells in the three is noticeable.   The central corpus luteum shows in the cavity an ovum whose envelope is crumpled.   The membrana granulosa is beginning to hypertrophy, and encroaches on the central cavity, particularly on the right hand side.

Plate xix.

Fig. 23.—A " corpus luteum atreticum " at about the stage represented in fig. 13.

Note the degenerated ovum towards the centre of the structure.   It is placed against the left wall of the largest vascular space.   Other vascular spaces lined by endothelium are also well seen, as well as the general similarity between this figure and fig. 13.

Plate xx.

Fig. 24 represents a " corpus luteum atreticum."   This figure should be compared with fig. 14.   The similarity in the structure of the two is noteworthy.

In the centre of the field is seen the remnant of the atrophied ovum invaded by leucocytes.   Round this the " corpus luteum atreticum " has formed, and is exactly similar to the " corpus luteum verum."   This figure illustrates the general dilatation of the blood vessels round the corpus luteum, and also the thinning of the ovarian stroma generally, with portions of corpora lutea on either side of the corpus luteum atreticum.